Addressing Free Radical Oxidation in Acne Vulgaris

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ABSTRACT

Objective: Comparatively little attention has been paid to the role of free radical oxidation in acne vulgaris. Here, using the traditional abnormalities cited for acne, the authors address the role of free radical oxidation throughout the pathogenesis by detailing the chemistry that may contribute to clinical changes. To probe the effects of free radical oxidation and test an antioxidant, they conducted a preliminary study of topically applied vitamin E. **Methods:** Seventeen patients with mild-to-moderate acne vulgaris were evaluated over an eight-week period in two private dermatology practices in this open-label study. All patients enrolled were on the same baseline regimen of salicylic acid and benzoyl peroxide. This regimen was then supplemented with topical vitamin E in sunflower seed oil. **Results:** At the end of the eight-week period, all patients demonstrated clinical improvement, as indicated by a reduction in the number of lesions and global mean difference. A statistically significant reduction was noted as early as Week 2. Enrolled patients also expressed a positive experience due to good tolerability and easy application. **Conclusion:** Although the exact pathogenesis of acne vulgaris remains unknown, the presence of excessive reactive oxygen species can be implicated in each of the major abnormalities involved. This presence, along with the positive results of the authors' preliminary study, demonstrates the need for more exploration on the use of topical antioxidants in limiting free radical oxidation in the acne model. This paper is designed to stimulate academic discussion regarding a new way of thinking about the disease state of acne. (*J Clin Aesthet Dermatol.* 2016;9(1):25–30.)

Acne vulgaris is the most common dermatologic condition in the United States and is a condition that has a multifactorial pathogenesis and an etiology that currently remains unknown.[1,2] Traditionally, it has been characterized by excessive sebum production, follicular epithelial hyperkeratosis, and rupture of follicular epithelium, resulting in an increased release of inflammatory mediating agents. Bacterial colonization (*Propionibacterium acnes*) has also been implicated in the pathology.[3,4] The above characterizing features of acne vulgaris represent deviations in processes that normally take place within the pilosebaceous units of healthy skin.

Previous studies have reported that oxidative stress components, such as reactive oxygen species (ROS) and lipid peroxide (LPO), may be involved in parts of the pathogenesis and progression of acne vulgaris.[2,5,7–11] During the formation of a ROS, oxygen will acquire an unpaired electron, thus forming a free radical. This free radical then has the ability to produce other ROS, such as peroxides, resulting in oxidative damage that may include lipid peroxidation and the secretion of inflammatory cytokines. The skin is especially susceptible to resultant oxidative damage, as it is chronically exposed to ROS-induced oxidative stress that is generated both from endogenous and exogenous sources.

Endogenous sources of ROS in the skin include auto-oxidation and enzymatic oxidation. Auto-oxidation involves a three-stage process: First, a free radical initiates the ultimate production of ROS, by extracting a hydrogen atom from a methylene group of polyunsaturated fatty acids.[5] This is known as the initiation stage. The propagation stage then occurs, in which the fatty-acid-lipid-peroxyl radical is able to extract hydrogen from another lipid molecule,

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particularly in the presence of heavy metals (i.e., zinc, copper, etc.), thus causing an autocatalytic chain reaction. The catalytic chain reaction is interrupted during the third stage, called termination, in which the lipid-peroxyl radical is reduced to a stable lipid peroxide. This is achieved via an antioxidant, such as alpha-tocopherol, in the skin's microenvironment.[5]

The other endogenous process through which lipid peroxides can form is via enzymatic oxidation. During enzymatic oxidation, lipoxygenase catalyzes the oxidation of polyunsaturated fatty acids, such as linoleic and arachidonic acids. Cycloperoxidases then catalyze polyunsaturated fatty acids and oxygen into endoperoxides, which are intermediates formed prior to conversion into prostaglandins. All enzymatic-produced free radicals are biologically active, thus an increased activity of the enzymatic oxidation process may result in an accumulation of ROS in the pilosebaceous units. This likely contributes to the abnormalities associated with acne vulgaris.[5–7,13] Excessive ROS may also be generated as byproducts of enzymatic peroxidation related to bacterial enzymes and the action of neutrophils and other immune response mechanisms triggered by the events occurring within the pilosebaceous unit.[5,9,12]

In addition to endogenous sources, photooxidation and pollutant/environmental oxidants also contribute to the pathogenesis of acne via the production of ROS. All unsaturated lipids are suspect to photooxidation in the presence of sensitizers, such as ultraviolet (UV) light. During photooxidation, a singlet oxygen (photooxidant type 2, or PO2) employs a direct attack by the electrophilic oxygen on the lipid chain at the site of the double bond.[5,10,12–13] This allows for the extraction of a hydrogen bond from a CH-group, rapidly initiating the propagation of molecule-to-molecule peroxidation. Both singlet (PO2) and triplet oxygen (PO1) species are generated by exposure to UV light, toxic molecules, and/or environmental factors. However, the PO2, or singlet oxygen, predominates. The generation of ROS then proceeds in a manner similar to auto-oxidation as explained above. The production of ROS, as initiated by free radicals during photooxidation is 1,000 to 1,500 times faster than auto-oxidation, as the former does not require an initiation step.[5,10,12–13]

The involvement of free radical oxidation (FRO) in each of the major abnormalities associated with acne has been suggested, as ROS and lipid peroxidation may play a role in the initiation and progression of epithelial inflammation in the pilosebaceous unit.[2,5,7–10] In acne, sebum composition is altered. Lipid peroxidation of fatty acids and unsaturated triterpenes, such as squalene, generates both intracellular and extracellular ROS. The generation of ROS can alter the viscosity and composition of sebum.[5,14] In fact, the excessive sebum production in acne is expected to promote the generation of excess ROS, leading to oxidative stress within the follicle, thus overwhelming the antioxidant system of the skin.[5,14]

The oxidants generated by lipid peroxidation are also suspected of promoting the formation of hyperkeratinous impactions. These powerful oxidants are capable of comedogenic potentiation.[5–6,14] Lipid peroxides can lead to the formation of advanced glycation end products (AGEs) via formation of byproducts, such as malondialdehyde (MDA), which can cross-link with proteins and alter the rigidity of keratin, thus enhancing follicular impaction. Could FRO also dictate the rate of appearance of acne lesions? Perhaps amino acid residues containing disulfides (cysteine) in peptide chains are more susceptible to cross-linking and plug formation. This is sporadic, but is certainly suggestive of a potentially continuous cycle, which exacerbates the clinical appearance of acne lesions.

Additionally, lipid peroxides can induce inflammatory responses in the follicles' walls, ultimately weakening the walls and making them more susceptible to rupture. ROS produced by neutrophils are also implicated in the irritation and destruction of the follicular wall. As a result of the destruction of the follicle wall, follicular contents are released into the surrounding tissue, resulting in a foreign body reaction.[13]

Oxidative stress in lipid peroxidation may also be an initiator for the release of inflammatory agents that contribute to the initiation of pathology.[6,7] Elevated pro-inflammatory factors, such as interleukin-1 (IL-1), have been noted to appear around clinically normal pilosebaceous follicles of acne patients prior to hyperproliferative and abnormal differentiation events.[7,8]

Under normal conditions, the antioxidant defense system of the skin prevents the formation of free radicals and ROS in pilosebaceous units, thus preventing oxidative injury of the structural lipids and proteins that maintain the integrity of the skin.[13] Non-enzymatic antioxidants, such as alpha-tocopherol (vitamin E) and beta-carotene, interact with free radicals, such as lipid peroxyl radicals, to prevent lipid peroxidation in the skin's microenvironment. However, in the presence of excessive lipid peroxidation and excess ROS, the redox balance (loss or gain of electrons) of the skin is offset, leading to a reduction in protective antioxidants, such as vitamin E.

Vitamin E is a lipid soluble antioxidant that neutralizes the oxidant effect of free radicals and thus prevents cellular damage.[15] High levels of vitamin E are found in sebum and sebum-rich areas, such as the upper layers of facial skin.[16,17] The sebaceous gland delivery of vitamin E may serve to protect the skin surface from oxidation, which may ultimately lead to the lipid peroxidation. However, when excess sebum is produced, the endogenous vitamin E supply may be overwhelmed, resulting in a reduction of this antioxidant and an increase in oxidative stress.

The influence of a high oxidative load on an overwhelmed antioxidant defense system is implicated in the pathogenesis of acne vulgaris. Studies have indicated that daily oral supplementation of alpha-tocopherol leads to significant increases of vitamin E levels in the human

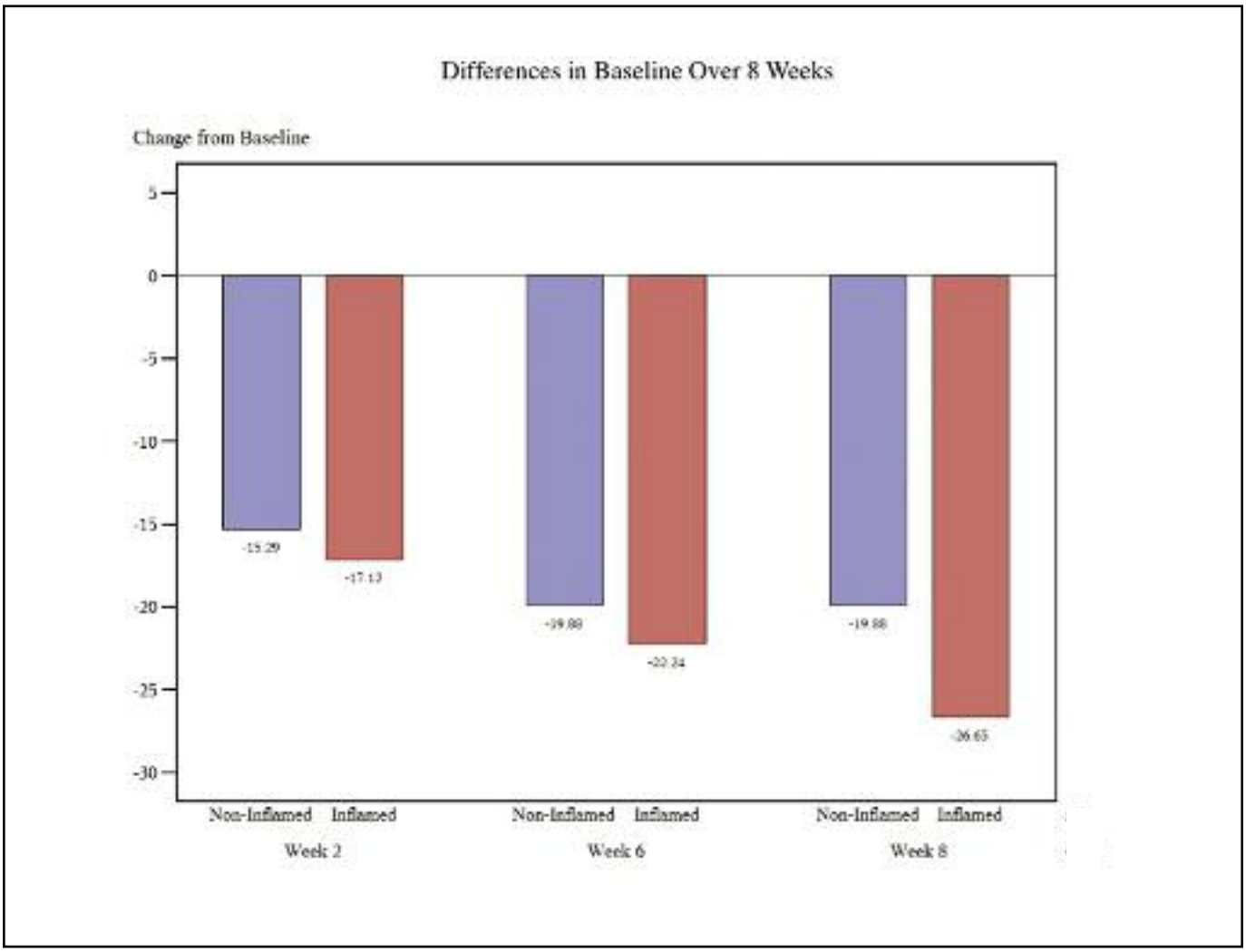


Figure 1. Combined benzoyl peroxide-salicylic acid and tocopherol results: Mean reduction in lesion count over eight weeks of treatment

skin, most notably at sites with a high density of sebaceous glands.[16] Additionally, research has indicated that serum vitamin E levels negatively correlate with the severity of acne vulgaris in patients.[18] Results from a UV erythema study demonstrated that pre-treatment of skin with topical alpha-tocopherol reduced UV-induced skin erythema in those with skin type II.[19] These results are attributed to vitamin E's antioxidant potential, in which the ROS that give rise to the post-UV-induced erythemal response were mitigated by the presence of vitamin E. Thus, the addition of vitamin E to current treatments for acne vulgaris may reduce the oxidative stress in the pilosebaceous unit and thus, decrease the ROS associated with acne vulgaris.

Since oral vitamin E can take weeks before affecting sebum, supplementation with topical vitamin E is often more appealing for treatment. The growing antibiotic resistance of *P. acnes* to systemic and topical antibiotics[20] has motivated dermatologists to look for alternative treatment options that decrease reliance on antibiotics, most notably benzoyl peroxide (BPO). Although undoubtedly beneficial, the use of BPO has also been demonstrated to reduce endogenous epidermal vitamin E by up to 95 percent, which is of much concern since the major route of delivery of vitamin E is via sebaceous glands. The supplementation of BPO with topical vitamin E has been suggested. Previous studies have indicated that the presence of vitamin E and other tertiary amines enhances the efficacy and tolerability of topical BPO.[21,22] Furthermore, it has been found that applying vitamin E in advance of BPO may lead to enhanced effects in the treatment of acne vulgaris, although more research is necessary.[22]

METHODS

In an attempt to investigate the potential clinical benefit of an antioxidant in offsetting the effects of oxidative stress, dermatologists at two private practices supplemented patients' current acne regimen of BPO and salicylic acid (SA) with tocopherol. All enrolled patients

TABLE 1. Statistical analysis test table

QUESTION	EVALUATION	N	MEAN	MEAN DIFFERENCE FROM BASELINE	WITHIN TREATMENT (SIGNED RANK) P-VALUE	WITHIN TREATMENT (T-TEST) P-VALUE
Non-inflamed	Baseline	17	31.59	–	–	–
	Week 2	17	16.29	-15.29	0.0001	0.0165
	Week 6	17	11.71	-19.88	<0.0001	0.0338
	Week 8	17	11.71	-19.88	0.0006	0.0340
Inflamed	Baseline	17	34.18	–	–	–
	Week 2	17	17.06	-17.12	<0.0001	0.0059
	Week 6	17	11.94	-22.24	<0.0001	0.0017
	Week 8	17	7.53	-26.65	<0.0001	0.0017
Macules	Baseline	17	27.18	–	–	–
	Week 2	17	27.76	0.59	>0.5000	>0.5000
	Week 6	17	26.35	-0.82	>0.5000	>0.5000
	Week 8	17	23.29	-3.88	>0.5000	0.3520
Global	Baseline	17	5.59	–	–	–
	Week 2	14	3.64	-1.75	0.0005	<0.0001
	Week 6	13	2.81	-2.58	0.0132	0.0038
	Week 8	10	2.45	-2.25	0.0039	0.0033

TABLE 2. Percent of patients experiencing adverse events after eight weeks of treatment

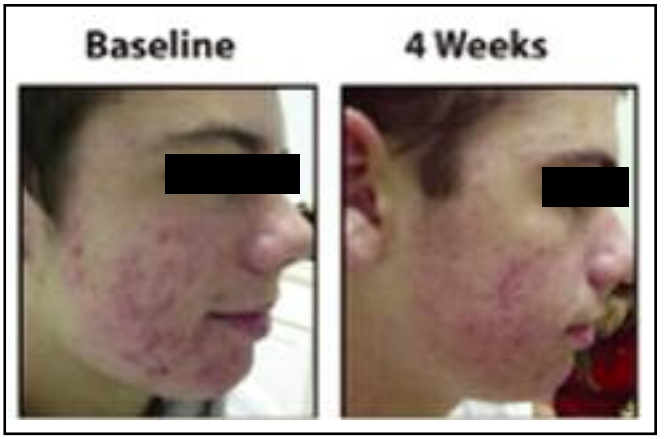
SIGNS AND SYMPTOMS	NONE	MILD	MODERATE	SEVERE
Erythema	50	30	20	0
Peeling	40	37	23	0
Burning	57	25	19	0
Itching	55	27	18	0
Stinging	57	22	20	0
Tightness	43	35	22	0

had mild-to-moderate acne vulgaris and were receiving baseline treatment with a daily comedolytic (1% or 2% SA) and a low strength antimicrobial (4% or 8% BPO), of which both were delivered via large pad delivery systems.

Seventeen patients with mild-to-moderate acne vulgaris were enrolled in this study. Mild-to-moderate acne vulgaris was defined using the global evaluation scale. The global evaluation scale consisted of 0 to 10, with 0 being none and 10 being severe. At baseline, the average global evaluation of patients' acne was 5.59.

All involved patients were seen over an eight-week period, during which they were required to supplement their current acne regimen with topically applied vitamin E [tocopherol 5% (alpha 11.4%, delta 15%, gamma/beta 45%) in sunflower seed oil]. The supplemented regimen was applied once per day over an eight-week period.

After written consent was obtained, survey information and photographs were taken during



Figures 2 and 3. Two different patients after four weeks of supplemented treatment with tocopherol

screening and then at Week 2, Week 6, and Week 8. The same investigator observed the patients during the course of the study. At each visit, a Patient and Physician Evaluation form was completed. The patient evaluation consisted of a subjective ranking of their complexion compared to their last visit and any changes in symptoms experienced. The physician's evaluation included a global evaluation of the patient's acne vulgaris on a scale of 0 (none) to 10 (severe), which includes assessment of erythema and size of all lesions. Both the lesion type and count were also documented. Noninflammatory lesions consisted of open and closed comedones, whereas inflammatory lesions included papules, pustules, and nodules, if present. Photographs of each patient were taken at each visit for comparison to baseline.

RESULTS

The results of the pilot study are shown in Figure 1. At baseline, the mean lesion numbers were non-inflamed (31.59), inflamed (34.18), and macules (27.16). As presented in Table 1, statistically significant reductions in the mean number of non-inflamed and inflamed lesions were noted as early as Week 2. The difference in mean global evaluation at Week 2 was also noted to be statistically significant, suggesting that the supplementation of the BPO and SA regimen with vitamin E produces substantial improvement in acne vulgaris. At the end of the eight-week period, there was a mean reduction of 19.88 for non-inflamed lesions (p value of 0.0006), 26.65 for inflamed lesions (p value of <0.0001), and 3.88 for macules (p value of >0.5). The global mean difference was -2.25, with a statistically significant p value of 0.0039.

Reported side effects ranged from mild to moderate. No severe side effects were noted. The mean percentage of each side effect is noted in Table 2.

DISCUSSION

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit and is considered to be the most prevalent skin disorder in the United States.[1] Acne pathogenesis is characterized by excessive sebum production, follicular epithelial hyperkeratosis, rupture of follicular epithelium, and colonization by *P. acnes*. As noted above, ROS can be implicated in each of the abnormalities associated with acne vulgaris. The skin's redox environment consists of chemical reactions that involve the transfer of electrons, often changing the oxidation state of atoms. When there is an imbalance in the skin's redox environment, oxidative stress may overwhelm the system, resulting in alterations in the function and molecular composition of the keratinocytes and sebocytes that make up the pilosebaceous unit. One such alteration may include increased membrane lipid peroxidation, which may ultimately evoke immune and inflammatory responses.[5,12] Treatment options that limit the presence of free radical oxidation in acne vulgaris, thus restoring the redox balance of the skin's microenvironment, may be beneficial as supplementation to currently recommended acne vulgaris treatments.

The positive results of the preliminary study featured in the author's paper demonstrates the need for a double-blind, randomized controlled trial to evaluate the efficacy of the addition of topical vitamin E to a regimen of SA and BPO in the treatment of acne vulgaris. The need for further research to support their preliminary findings is imperative, given the limitations of the study included in this paper. The first limitation of this study was the small sample size. The second limitation of this study was the open label protocol without control, thus precluding the elimination of sampling bias. Additionally, the lack of vehicle control prevented the elimination and isolation of confounding variables and bias. Thus, the results of this study must be analyzed with caution. Although the limitations of this study must be considered, the advantages of the study must also be taken into consideration. For one, all subjects enrolled in this study were on the same baseline treatment of SA and BPO. Secondly, the global and lesion data were subjected to

statistical analysis, which together with the photographs, demonstrate the validity of positive clinical changes.

Thirdly, while considering the limits of this study, the dermatologists involved remain confident that the study points to worthwhile findings. Like other dermatologists in private practice, they customarily evaluate new treatments in diverse patient populations using their expertise and experience without blinding, randomized controlled or large number clinical studies. These evaluations become the basis for deciding the role of the modality in their practice.

The positive results were intriguing, as all patients demonstrated a decrease in the global evaluation score of the severity of their acne over the eight-week period. Additionally, there was a significant reduction in the number of noninflammatory and inflammatory lesions, of which was noted as early as Week 2. A decrease in both lesion inflammation and generalized erythema, which sometimes occurs between lesions, was also noted for all patients by the first follow-up visit at Week 2 as well. The regimen was noted to be cosmetically acceptable with easy application and few mild-to-moderate side effects. We believe that these features, along with the positive results, contributed to the excellent patient compliance.

The preliminary findings of the clinical study featured in the authors' paper demonstrate the need for further research on the relationship of FRO in the pathogenesis of acne vulgaris. By better understanding the potential role of FRO in the acne model, additional treatment options, such as topical antioxidants, may be utilized to further limit the presence of oxidative stress in the pilosebaceous unit.

The authors hope this paper will not only expand existing knowledge, but will also stimulate academic discussions about a new way to think about the disease state of acne vulgaris.

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